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Stimulation, toxicity, and antagonism of calcium nitrate and manganese chloride as indicated by growth of wheat roots

LELA V. BARTON AND SAM F. TRELEASE

(WITH THREE TEXT-FIGURES)

The importance of certain chemical elements, such as calcium,[1] as necessary for normal growth of higher plants has long been known, but it is only comparatively recently that detailed investigations have been made to determine the quantities of manganese, boron, and zinc that may be indispensable for normal growth of plants in general or of certain plants in particular. It was shown very early that salts of these and of many other elements may have a poisonous action upon plants, but it was also reported by various investigators that solutions containing extremely low concentrations of the salts of these and of several other elements seem to be beneficial rather than harmful to certain plants. The beneficial effects have generally been termed stimulating effects and may be expressed by the plant in various ways, such as by more rapid increase in length of tops or roots, greater luxuriance of foliage, greater dry weight, greater green weight, etc. Brenchley (1914) gives a number of interesting cases of such stimulation. A survey of the literature, however, would not warrant the generalization that every toxic substance acts as a stimulant when supplied in a sufficiently dilute solution.

The powerful effect of zinc salts in minute traces on the growth of fungi was discovered by Raulin (1869, 1870). Raulin also observed a striking effect of manganese salts, but he was doubtful whether the effect of the manganese salts was not due to traces of zinc. Richards (1897, 1899) tested a number of ele-

[1] The ten elements whose necessity for ordinary green plants was established very early are: K, Ca, Mg, Fe, N, S, P, C, O, H. With the exception of iron, these are required in relatively large amounts, and it is easily demonstrated that green plants cannot develop if any one of these elements is not supplied.

[The BULLETIN for June (**54**: 471–558) was issued 2 September 1927.]

ments and concluded that the stimulating action of zinc in increasing the dry weight of fungi is shared with other elements, such as iron, lithium, cobalt, etc. These general results, especially with zinc, have been confirmed in a number of other investigations cited by Steinberg (1919), as well as by his own results.

A remarkable case of the effect of manganese is seen in the work of Bertrand and Javillier (1912a), who report that one part of manganese in one million of the culture solution increased slightly the dry weight of *Aspergillus*, and the effect continued to increase even up to one part in 100. In another test they found (1912b) that a combination of manganese and zinc was more effective than either alone. An extraordinary effect of zinc is reported for one experiment which showed that a definite increase in yield was produced by the addition of one part of zinc to twenty-five million parts of solution, and one part in ten millions nearly doubled the yield.

The evidence is not conclusive as to whether infinitesimal amounts of certain of these elements are absolutely indispensable to the normal physiological activity of the fungus protoplasm or whether minute traces of these elements, though non-essential, are stimulating in their action. The former view is held by Bertrand and Javillier (1912a, 1912b), who regard traces of manganese and zinc as essential for normal metabolism. Richards (1897, 1899), on the other hand, has emphasized the stimulating effects. Richards points out that stimulated cultures have a heavily matted mycelium and exhibit diminished spore production. It is possible that extremely small quantities of zinc and manganese are essential for normal growth and that slightly larger amounts give greater growth—the latter accompanied, however, by the growth modifications described by Richards. A double action of this sort is reported by Richards for iron salts.

Manganese salts, in small quantities, appear to have important beneficial effects on the growth of the higher green plants. Although larger quantities of manganese have a toxic influence on growth, recent work indicates that minute traces of this element may be essential for normal growth and maturation.

McHargue (1914) made a study of the manganese content of the seed coats of various seeds and considered that the presence

of manganese in the seed coats indicated a relation of this element to the oxidizing enzymes which not only have to do with the selection, making, and storing of food in the kernel, but which may also stimulate the enzymes which break up fats, sugars, and starches, making them available as food for the young seedling.

In later papers McHargue (1919, 1922, 1923, 1924, 1925) has presented evidence, from actual cultures of plants deprived of manganese and of those supplied with traces of this element, that manganese is indispensable for the normal growth and development of ordinary green plants.

Leguminous plants were found by McHargue (1922) to be more sensitive than other plants to a lack of manganese, and he concluded that this element is apparently concerned in the assimilation of nitrogen and the synthesis of protein. He thinks that manganese plays the rôle of a necessary catalyst in plant metabolism, and that manganese, together with iron, functions in the synthesis of chlorophyll. References to the earlier literature on the effects of manganese are given in one of the papers by McHargue (1923).

Brenchley (1914) cites many investigations in which beneficial effects of manganese have been obtained with soil cultures. She also reports one experiment (Brenchley, 1910) in which barley was grown to maturity in a series of water cultures containing nutrient solutions to which manganese sulphate was added. In a later series of tests the plants were allowed to grow only seven weeks. In both cases, manganese sulphate in minute traces had a decided stimulating action on root and top growth. A discrepancy in results of the two experiments Brenchley attributed to a difference in the concentration of the nutrient salts employed and to a difference in length of growing period.

It has recently been found that a form of chlorosis of spinach and beets is associated with smaller amounts of manganese compounds in the chlorotic tissue than in the healthy tissue, and that the chlorosis could be cured by spraying a dilute solution of manganese salts on the leaves or by mixing these salts with the soil (McLean and Gilbert, 1925; Gilbert, McLean, and Hardin, 1926; Gilbert, McLean, and Adams, 1927).

A survey of the literature indicates that most of the work on the effect of manganese on the higher plants has been done with

soil cultures and with solution cultures containing complex mixtures of salts. Relatively little attention has been given to the effects of dilute simple solutions of manganese salts, especially with reference to the possibility of obtaining an acceleration of growth with such solutions. The effects of single-salt solutions of manganese should be of special importance as representing conditions considerably less complex than those involved in soil cultures or in mixed solutions containing four or more nutrient salts.

Recent work has indicated that minute traces of several elements besides manganese may be essential for the growth of green plants. Somner and Lipman (1926) have furnished photographic evidence that normal growth and development of a considerable number of widely different, higher green plants did not occur when boron and zinc were excluded from the culture solution. Brenchley and Warington (1927) say that normal growth of certain plants does not occur when the culture solution lacks boron and that no other element, out of fifty-two tested, has proved capable of replacing boron. Special attention was given to manganese, the necessity of which had previously been determined.

The important effects of manganese which have been reported by various investigators emphasize the need of further detailed studies of the influence on plant growth of manganese salts, alone and in combination with other salts.

That the toxicity of manganese salts may be decreased or even eliminated altogether by addition of calcium salts, has been established. McCool (1913) investigated the toxicity of manganese salts to green plants and the antagonistic relations between salts of manganese and of other elements. In one test, in which only ten Canada field pea seedlings were used, he found that a simple solution of 0.000125 M manganese chloride was injurious and that a similar solution containing 0.00025 M manganese chloride prevented growth entirely. This test was continued for five days. In other tests with Canada field pea seedlings he found that the poisonous action of manganese salts toward the pea seedlings was greatly reduced when the usual nutrient salts were present in the culture solution, the lethal concentration of manganese chloride in the latter case being four hundred times as strong. From the results of these and other

experiments, he concluded that simple solutions of manganese salts are extremely poisonous to pea and wheat seedlings, but the degree of toxicity is much lower when manganese salts are added to nutrient solutions or to soil cultures.

The injurious action of solutions of manganese salts was found by McCool (1913) to be manifested mainly toward the tops of the plants, chlorosis of the leaves being the first indication of an injurious concentration of a manganese salt. It is interesting to note that manganese salts were found to be less injurious to plants grown in the dark than to those grown in the light. Calcium, potassium, sodium, and magnesium salts were each effective in counteracting the poisonous action of a manganese salt. Mutual antagonism was observed to exist between a manganese salt and salts of each of the following elements: potassium, sodium, and magnesium. But in no case did the addition of a manganese salt to a solution of a calcium salt result in an increased development of plants over that in the corresponding simple solutions of the calcium salt.

The present paper reports experiments upon the elongation of wheat roots in simple solutions of different concentrations of manganese chloride and calcium nitrate and in mixed solutions containing combinations of the two salts in different proportions. The objects of this study were: (1) to determine whether stimulating effects could be observed in simple solutions of manganese chloride or of calcium nitrate; and (2) to determine the possible antagonistic effects of these two salts in mixed solutions.

METHOD

The method of procedure in performing each series of experiments, the results of which are given in the ensuing pages, was essentially the same as that described in papers by Trelease and Trelease (1925, 1926a, 1926b).

Large and small beakers were used, all of which were thoroughly cleaned and then rinsed in tap water, after which they were dipped in hot distilled water and hung on glass rods to dry. After drying, they were removed and again dipped in hot distilled water and replaced as before. The germinating dishes, graduated cylinders, flasks, and all other glassware, as well as forceps and scissors, were treated in the same manner.

Molecular solutions were prepared by dissolving weighed

amounts of analyzed salts in water obtained from a Barnstead still. The concentrated solutions were diluted and mixed to obtain the desired concentrations and proportions.[2] In every series two cultures supplied with a three-salt standard solution and one with distilled water were used as controls. The three-salt control contained 0.02 M potassium acid phosphate, 0.02 M calcium nitrate, and 0.02 M magnesium sulphate.

The culture solutions were transferred to the beakers which had previously been prepared in the following manner: The mouths of the small beakers (300 cc. Pyrex, tall form, without spout) were covered with paraffined bobbinet, which had previously been boiled in distilled water. A paraffined linen thread was used to tie the bobbinet over the beaker. This having been done, the bobbinet was trimmed and each small beaker was placed inside a larger one (600 cc.) of the same form.

The solutions were then poured in until the small beaker was entirely filled, and the solution filled the space around it, the level being even with the top of the small beaker, so that no air bubble was left under the bobbinet. Care was taken that the level of the solution did not extend above the bobbinet. Duplicate cultures with each solution were prepared.

In the bottom of each of five glass-covered culture dishes (24 cm. in diameter) were placed sheets of filter paper, which were then moistened with distilled water. Into the dishes were scattered the wheat grains (Marquis wheat supplied by Julius Schendel, Campbell, Minn., 1924 crop), which had previously been soaked in distilled water for three hours and rinsed several times to remove foreign matter which might be present. The grains were taken individually with forceps and placed furrow side down. The dishes were left at ordinary room temperature until the primary root had attained a length of about 6 mm. (usually 24 to 28 hours).

The germinated grains were then placed on the bobbinet at the surface of the solutions, so that the primary roots dipped into the solutions. Before each grain was placed in contact with the first solution, the length of the primary root was recorded. Then a grain with a primary root of approximately the same

[2] Volume-molecular concentrations are used in this paper, each value for concentration representing the number of gram molecules contained in each liter of solution.

length was placed in each of the twenty-one other cultures. This process was repeated until there were twenty-five actual measurements of primary root lengths. An average of these lengths was obtained as a measurement of the initial root length.

The cultures were then placed in a dark chamber (described by Trelease, 1925), which was kept at approximately 19° C. During the first forty-eight hours, each culture was covered with a watch glass.

The duration of the growth period was the length of time it took the primary roots of the three-salt control to elongate about 80 mm. (from 6 mm. to 86 mm.), the period thus required being approximately 100 hours. The cultures were then removed from the incubator and observations made on the general appearance of the roots of the plants of each. The length of the primary root of each seedling was recorded. Each of the growth values given in the tables was obtained by deducting the mean initial root length from the mean final root length, and expressing this value for elongation as a percentage of the average elongation for the standard three-salt solution.

RESULTS AND DISCUSSION

Single-salt solutions

It is of special interest to note the relations between growth rates and volume-molecular concentrations of manganese chloride and calcium nitrate in single-salt solutions. These data are given in table 1 and are plotted in the graphs of figure 1. In these graphs, as well as those of figures 2 and 3, the ordinates represent elongation expressed as a percentage of that for the three-salt standard solution, and the abscissas represent concentration (plotted on a logarithmic scale, except the zero concentration).

Stimulation of growth by simple solutions of manganese chloride. It will be noticed from the graph of figure 1 that there was in the case of manganese chloride a marked stimulation of growth in certain concentrations. Wheat roots grown in 0.00001 M manganese chloride show an elongation of over forty per cent greater than that attained by roots in distilled water. This amounts to eight per cent greater elongation than that of the roots in the three-salt standard solution. In somewhat higher concentrations

of this salt, as 0.00005 M and 0.00008 M, the elongation continues to be more rapid than in distilled water, but it is less rapid than in the standard solution. More concentrated solutions than these, however, cause the growth curve to drop very

TABLE I

Growth of wheat roots in single-salt solutions of manganese chloride and of calcium nitrate; growth values are expressed as percentage of growth for the three-salt standard solution[1]

VOLUME-MOLECULAR CONCENTRATION, M	MANGANESE CHLORIDE			CALCIUM NITRATE			
	A	B	Average	A	B	C	Average
0	—	77[2]	75[3]	82[2]	68[2]	84[2]	75[3]
0.0000001	—	—	—	79	76	81	79
0.000001	—	—	—	79	69	74	74
0.00001	103	112	108	86	82	84	84
0.00005	94	83	89	—	—	—	—
0.00008	83	78	81	—	—	—	—
0.0001	—	—	—	84	61	69	71
0.000126	34	38	36	—	—	—	—
0.0002	25	22	24	—	—	—	—
0.00032	15	14	15	—	—	—	—
0.0005	7	11	9	—	—	—	—
0.0008	5	7	6	—	—	—	—
0.005	2	2	2	—	—	—	—
0.001	—	—	—	54	45	58	52
0.01	—	—	—	35	35	37	36
0.1	—	—	—	27	20	22	23

[1] Each value is the average of duplicate cultures of 25 seedlings. Thus the final average for manganese chloride represents 100 seedlings; that for calcium nitrate, 150 seedlings.

[2] Value obtained in this particular series.

[3] Average for all distilled water cultures (25 seedlings each): 76, 76, 74, 69, 81, 78, 67, 65, 72, 72, 77, 84, 65, 66, 76, 88, 97, 94, 76, 76, 67, 67, 61, 66, 83, 86.

quickly, until 0.005 M allows only about three per cent of the growth for distilled water.

The results secured in the present experiments appear to offer conclusive evidence that simple solutions of manganese chloride have a very pronounced effect in stimulating root elongation. They serve therefore to extend the earlier observations which showed stimulating action on older plants growing in soil cultures or in mixed nutrient solutions (Brenchley, 1910; Loew and

Sawa, 1902; Aso, 1902). The presence of nutrient salts in the culture solution is apparently not essential to the growth-accelerating effect of manganese.

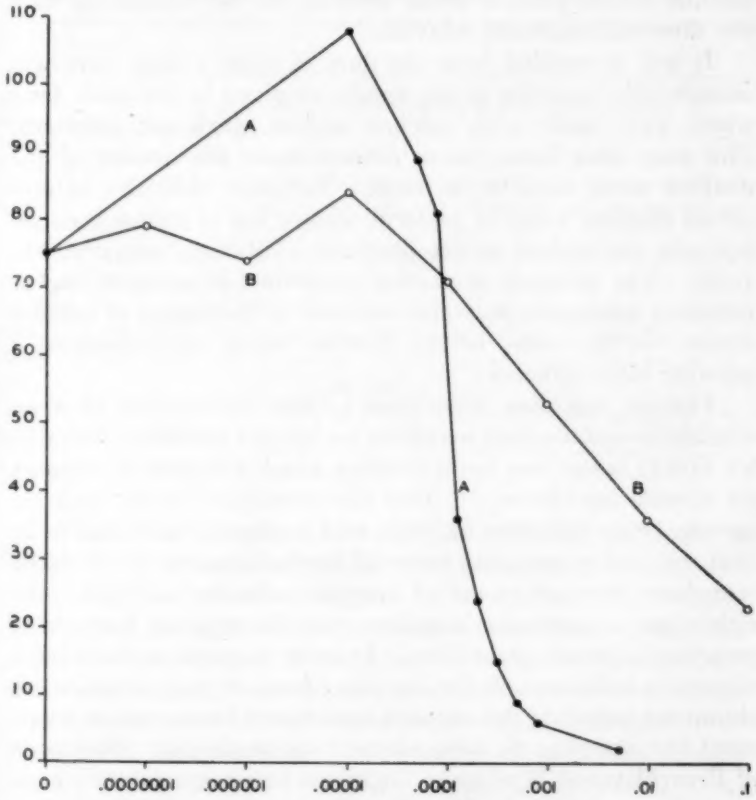

FIG. 1. Growth of wheat roots in single-salt solutions of manganese chloride (*A*) and of calcium nitrate (*B*).

Ordinates represent elongation as percentage of elongation for three-salt standard solution. Abscissas represent volume-molecular concentration (logarithmic scale, except the zero concentration).

Growth in simple solutions of calcium nitrate. The graph for calcium nitrate (figure 1) is very different from that for manganese chloride, since it exhibits no pronounced rise indicative of stimulation. There is a suggestion, however, that certain concentrations of calcium nitrate may possibly allow slightly more rapid elongation than distilled water. Further study would

be needed to obtain conclusive evidence on this point, since the observed effect is so small that it may lie within the limits of the experimental error. In no case did a single-salt solution of calcium nitrate prove a better medium for root elongation than the three-salt standard solution.

It will be noticed from the data in table 1 that there was considerable variation in the results obtained in the three tests which were made with calcium nitrate single-salt solutions. This may have been due to differences in the quality of the distilled water used in the tests. The great difficulty of preparing distilled water of uniform quality has of course been repeatedly emphasized in the literature (Hibbard, 1915; Scarth, 1924). The presence of minute quantities of antagonizing or retarding substances may alter the rate of absorption of calcium nitrate by the roots, or the distilled water may excercise a separate toxic influence.

Theories regarding stimulation. Clear distinctions between stimulants and nutrient materials are not yet possible. Brenchley (1914) states two main theories which are held to account for stimulating effects: (1) That the stimulants act as catalytic agents, being valueless on their own account, but valuable in that they aid in procuring essential food substances; (2) that the stimulants themselves are of integral value for nutrition. In either case, a particular stimulant may be essential for normal growth of a certain plant form. In order to prove that a certain element is indispensable for any plant form, it must of course be shown not only that this element is necessary for normal development but also that no other element can replace it. The work of Brenchley and Warington (1927) on boron is especially convincing, since fifty-two other elements were tested and none was capable of replacing boron.

The effect of manganese in accelerating the rate of root elongation in very young seedlings may be quite different in nature from that which may render this element necessary for normal growth and maturation of green plants. At least, salts of certain elements known to be essential (calcium, potassium, and magnesium) may lack such stimulating action on root growth; simple solutions of the salts of these elements have generally been found to allow less rapid growth than distilled water (Trelease and Trelease, 1926a).

TABLE 2

Growth of wheat roots in nine series of mixed solutions containing manganese chloride and calcium nitrate; growth values represent each of the duplicate cultures, and are expressed as percentage of growth for the three-salt standard solution[1]

VOLUME-MOLECULAR CONCENTRATION OF CALCIUM NITRATE, M	VOLUME-MOLECULAR CONCENTRATION OF MANGANESE CHLORIDE, M															
	0.00001		0.00005		0.00008		0.000126		0.0002		0.00032		0.0005		0.0008		0.005	
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b	8a	8b	9a	9b
0	105	100	97	92	82	84	28	41	25	26	19	11	4	10	5	5	2	2
0.0000001	99	102	94	94	76	80	54	47	15	20	9	14	4	8	5	5	1	2
0.000001	102	93	85	88	76	78	58	59	52	50	11	15	7	7	6	5	2	2
0.00001	88	98	70	70	64	64	64	58	52	52	46	52	41	40	12	14	3	2
0.0001	92	86	59	62	47	40	47	51	43	43	45	47	47	52	50	48	16	16
0.001	52	59	54	55	56	59	49	51	43	45	41	43	34	37	39	35	27	29
0.01	38	40	35	35	37	42	45	42	40	43	39	39	37	33	41	42	25	26
0.1	29	29	29	31	29	29	28	29	26	28	30	33	28	31	31	26	15	16

[1] Each value represents 25 seedlings.

What some investigators have called stimulation might in certain cases be regarded as due to an antagonism to toxic substances in the distilled water used. For example, Scarth (1924) found that *Spirogyra* lived only from 5 to 20 hours in pure distilled water condensed in silica or platinum and having a pH value of about 5.5; however, the addition to the water of a suitable concentration of any one of many single salts greatly extended the duration of life—to as many as 528 hours in some cases. This extension of the life period he attributed to an antagonistic action of the salt to the toxicity of the water, the latter toxicity resulting, he says, from the hydrogen-ion concentration due to dissolved carbon dioxide. However, the acceleration of root growth in dilute solutions of manganese chloride can hardly be ascribed to antagonism of substances already present in the distilled water; for calcium nitrate, which has generally been found to be one of the most effective antagonizing substances, gave little or no evidence of growth acceleration, while manganese chloride—which usually has only a slight antagonistic action—gave a striking acceleration of growth.

Two-salt solutions

The data pertaining to root elongation in mixed solutions containing manganese chloride and calcium nitrate are given in tables 2 and 3 and are shown graphically in figures 2 and 3. In each of the nine series of cultures the manganese chloride content was held constant and the calcium nitrate was varied from zero to 0.1 M. The growth occurring in a solution of manganese chloride alone is shown by the first point in each curve, and the growth in simple solutions of calcium nitrate of the concentrations used in the mixed solutions is shown by a separate curve. The figures therefore show clearly (1) the growth in every mixed solution, (2) the growth in the corresponding simple solution of manganese chloride, and (3) the growth in the corresponding simple solution of calcium nitrate.

TABLE 3

Growth of wheat roots in nine series of mixed solutions containing manganese chloride and calcium nitrate; growth values represent the fifty seedlings in the duplicate cultures, and are expressed as percentage of growth for the three-salt standard solution[1]

VOLUME-MOLECULAR CONCENTRATION OF CALCIUM NITRATE, M	VOLUME-MOLECULAR CONCENTRATION OF MANGANESE CHLORIDE, M								
	0.00001	0.00005	0.00008	0.000126	0.0002	0.00032	0.0005	0.0008	0.005
0	*103*	*94*	*83*	34	25	15	7	5	2
0.0000001	101	*94*	78	50	18	11	6	5	2
0.000001	98	86	77	58	51	13	7	6	2
0.00001	93	70	64	*61*	*52*	*40*	40	13	3
0.0001	89	60	44	49	43	47	*49*	*40*	16
0.001	56	55	57	50	44	42	36	37	*28*
0.01	39	35	39	43	41	39	35	42	26
0.1	29	30	29	29	27	31	30	29	15

[1] The maximum in each vertical column is italicized.

Comparing the graphs of figure 2 with those of figure 3, it is seen that the form of the growth curves undergoes a distinct change as the concentration of manganese chloride becomes lower. The curves for the series containing the six highest concentrations of manganese chloride (figure 3) rise to a maximum value and then fall. Those for the three lowest concentrations (figure 2) have an entirely different form, since they fall continuously in passing from left to right.

Antagonism as indicated by diminished toxicity of manganese chloride. For the mixtures containing the six highest concentrations of manganese chloride (figure 3) it seems safe to consider that antagonism is shown in the usual way. Since each of these curves rises as the concentration of calcium nitrate increases, it appears to be reasonable to interpret this rise as due to the effect of calcium nitrate in antagonizing the toxicity of manganese chloride (Osterhout, 1918; McCool, 1913; Hawkins, 1913; Stiles and Jörgensen, 1914). This effect is clearly shown in each of the six curves. If, for each series, the increased growth due to antagonism is divided by the growth in the corresponding manganese solution, it will be noted that the greatest antagonism, by this criterion, occurs in the solutions in which manganese chloride is most concentrated.

The curve for calcium nitrate (dotted line) lies in general much higher than those for the mixtures. Mixtures having relatively high concentrations of calcium nitrate, however, gave slightly better growth than the corresponding simple solutions of calcium nitrate.

Antagonism as indicated by diminished stimulation by manganese chloride. For the mixtures containing the lowest concentrations of manganese chloride (figure 2), antagonism is not so readily defined. These mixtures contain concentrations of manganese chloride that in simple solution would stimulate root growth, in the sense that they would allow more rapid root growth than occurs in distilled water. And for these concentrations of manganese chloride the addition of successively higher concentrations of calcium nitrate decreases rather than increases growth. Thus the calcium nitrate appears to interfere with the stimulating effect of the manganese chloride, possibly by making the small amount of manganese chloride unavailable for use by the roots. Antagonism appears to be shown by an inhibition of the stimulating effect that low concentrations of manganese chloride would exhibit in simple solution. It will be observed that in four of the mixed solutions containing 0.00001 M manganese chloride and in two of those with 0.00005 M manganese chloride, the growth is more rapid than in the corresponding calcium nitrate solutions (dotted line).

Quantitative measurement of antagonism. It is evident that an exact quantitative measurement of antagonism can only be

obtained when it is possible to estimate the total toxicity that the two-salt solutions would have if neither salt influenced the

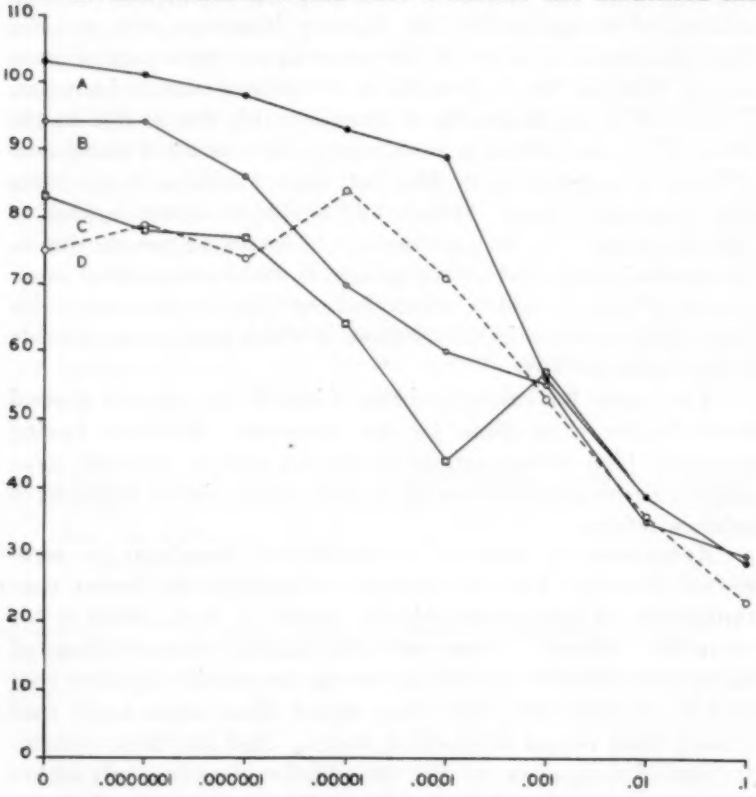

FIG. 2. Growth of wheat roots in two-salt solutions of manganese chloride and calcium nitrate.

Ordinates represent elongation as percentage of that for three-salt standard solution. Volume-molecular concentrations of calcium nitrate are shown by the abscissas (logarithmic scale, except the zero). *A* represents mixed solutions containing 0.00001 M manganese chloride; *B*, 0.00005 M; *C*, 0.00008 M. *D* (dotted line) shows growth in single-salt solutions of calcium nitrate. In each series the concentration of manganese chloride remained constant while that of calcium nitrate varied in the manner shown by the abscissas.

toxicity of the other salt. How this additive effect may be estimated has been shown by Osterhout (1914a, 1914b, 1915, 1918, 1922). The fundamental assumption necessary here is that the effect of one salt can be expressed in terms of the other

salt. This can be done if the curves for growth in simple solutions have nearly the same slope when a logarithmic scale is used for concentrations (Trelease and Trelease, 1926a). It seems inadvisable to attempt to estimate additive effects if the

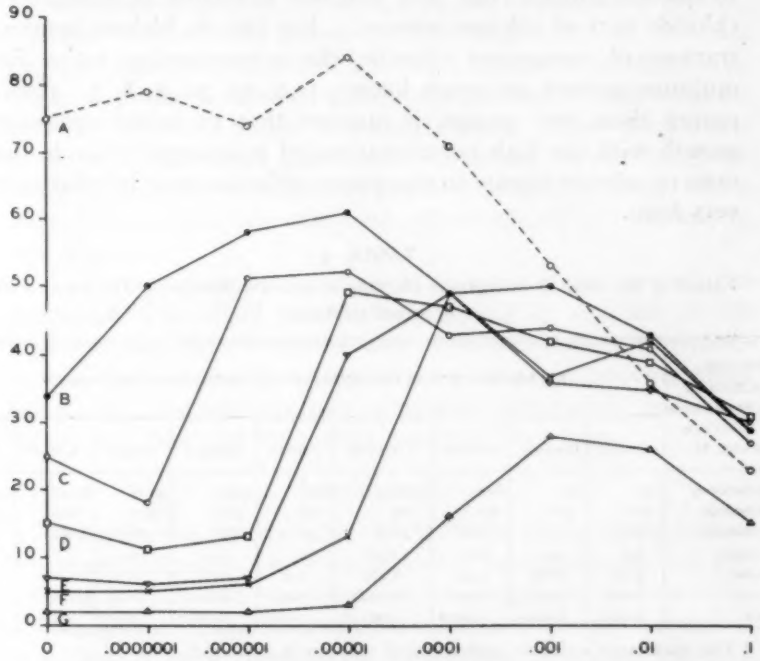

FIG. 3. Growth of wheat roots in two-salt solutions of manganese chloride and calcium nitrate.

Ordinates represent elongation as percentage of that for three-salt standard solution. Volume-molecular concentrations of calcium nitrate are shown by the abscissas (logarithmic scale, except the zero). *A* (dotted line) shows growth in single-salt solutions of calcium nitrate. *B* represents mixed solutions containing 0.000126 M manganese chloride; *C*, 0.0002 M; *D*, 0.00032 M; *E*, 0.0005 M; *F*, 0.0008 M; *G*, 0.005 M. In each series the concentration of manganese chloride remained constant while that of calcium nitrate varied in the manner shown by the abscissas.

curves differ in slope as much as do those for manganese chloride and calcium nitrate (see figure 1).

Molecular ratios of manganese chloride to calcium nitrate. The ratios of manganese chloride to calcium nitrate, especially those ratios allowing greatest root elongation, as shown in table 4,

furnish interesting data when examined in connection with the growth curves of figures 2 and 3. For the three lowest concentrations of manganese chloride best growth was secured in each case with the highest of the tested ratios of manganese chloride to calcium nitrate (100, 500, and 800 molecules of manganese chloride to 1 of calcium nitrate). For the six highest concentrations of manganese chloride the corresponding ratios for optimum growth are much lower—12.6, 20, 32, 5, 8, 5. Comparing these two groups, it appears that to secure optimum growth with the high concentrations of manganese chloride the ratio of calcium nitrate to manganese chloride must be relatively very high.

TABLE 4

Values of the ratio of manganese chloride to calcium nitrate for the nine series of mixed solutions[1]

VOLUME-MOLECULAR CONCENTRATION OF CALCIUM NITRATE, M	VOLUME-MOLECULAR CONCENTRATION OF MANGANESE CHLORIDE, M								
	0.00001	0.00005	0.00008	0.000126	0.0002	0.00032	0.0005	0.0008	0.005
0.0000001	*100*	*500*	*800*	1260	2000	3200	5000	8000	50,000
0.000001	10	50	80	126	200	320	500	800	5,000
0.00001	1	5	8	*12.6*	*20*	*32*	50	80	500
0.0001	0.1	0.5	0.8	1.26	2	3.2	*5*	*8*	50
0.001	0.01	0.05	0.08	0.126	0.2	0.32	0.5	0.8	*5*
0.01	0.001	0.005	0.008	0.0126	0.02	0.032	0.05	0.08	0.5
0.1	0.0001	0.0005	0.0008	0.00126	0.002	0.0032	0.005	0.008	0.05

[1] The maximum value in each vertical column is italicized.

A progressive change in the optimum proportions does not persist, however, within the series having the six highest concentrations of manganese chloride; for within these series the ratios for optimum growth (12.6, 20, 32, 5, 8, 5) agree as nearly as possible, considering the great differences in values of the consecutive ratios that were tested. A further, detailed study would be needed before very precise statements could be made concerning optimum proportions of manganese chloride to calcium nitrate.

It is of interest to observe in figures 2 and 3 the minimum concentration of calcium nitrate that appeared to have a decided effect on each of the growth curves, and to note in table 4 the corresponding molecular ratios. This examination shows that

one molecule of calcium nitrate to every fifty or one hundred molecules of manganese chloride may be expected to have a distinct influence upon the way in which the manganese chloride affects the plants.

Appearance of roots. Throughout the experiments the appearance of the roots grown in both single-salt and two-salt solutions indicated that each salt has a specific toxic effect. In response to the toxicity of manganese chloride, brown rings or splotches appeared on the very brittle roots. Often there was a series of brown rings distributed at rather regular intervals along the root and separated by apparently healthy tissue. It is difficult to explain why the toxic effects should be localized in this manner.

Calcium nitrate, in the highest concentrations, caused the formation of bands of transparent material on each side of the root and extending from the base to within one or two millimeters of the tip. In calcium nitrate solutions favoring rapid elongation, the roots became somewhat attenuated and very tender and provided with dense masses of root hairs.

SUMMARY

A study was made of the elongation of the primary root of wheat seedlings in single-salt solutions of manganese chloride and calcium nitrate, and in solutions containing both of these salts.

Manganese chloride, in simple solution of proper concentration, had a very pronounced stimulating effect on the root elongation of very young wheat seedlings.

Calcium nitrate, in dilute solution, did not appear to have a significant stimulating effect; it allowed about the same elongation as occurred in distilled water. At high concentrations calcium nitrate was found to be far less toxic than manganese chloride.

When growth in single-salt solutions is plotted against logarithms of concentrations, the slope of the graph for calcium nitrate is markedly different from that of the graph for manganese chloride; hence equally toxic solutions of these salts do not remain equally toxic when diluted to the same degree.

Calcium nitrate had a marked effect in reducing the toxicity that higher concentrations of manganese chloride showed in simple solution, and it also reduced the stimulating effect that

low concentrations of manganese chloride exhibited in simple solution.

The effect of calcium nitrate in diminishing the toxic action of manganese chloride appears to be much more pronounced than the effect of manganese chloride on the toxicity of calcium nitrate.

Differences in appearance of roots grown in single-salt solutions indicating specific toxicity were observed. The symptoms of poisoning by manganese chloride are especially interesting, since these are characterized by brown rings distributed at rather regular intervals on the root.

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Magnolia cordata and other woody plants

W. W. Ashe

MAGNOLIA CORDATA Michx.[1] For more than one hundred years after this tree was collected by Michaux and through him introduced into cultivation it was unknown in a wild state. Its rediscovery in 1913 by Mr. L. A. Berckmans in eastern Georgia growing near one of the localities from which originally reported by Michaux gives hope that *Gordonia altamaha* may eventually be found also. The magnolia was later reported by Berckmans from localities in middle Georgia. During the summer of 1926, it was found by the writer along Bear Creek, about 12 miles southeast of Tuscaloosa, Tuscaloosa County, Alabama, associated with *M. acuminata*, the oaks and hickories. It was also found, more abundant and of larger size, up to 70 feet tall, upon sandy soils, in hollows, and along small streams tributary to Kintabush and Yantley creeks, Choctaw County, Ala. Since these streams head in Mississippi, within 20 miles of where *M. cordata* is very abundant, it and also *Quercus arkansana* var. *caput-rivuli*, referred to below, should be looked for in that state in the hilly sandy region which lies to the west and south of Meridian.

Greene proposed three species of *Polycodium* from Florida—*P. caesium*,[2] *P. revolutum*[2] and *P. oliganthum*.[3] He noted that the bracts of all were often foliaceous, that consequently some of the flowers seemed axillary, and that the flowers were scattered over the plants in place of being largely clustered upon branches which seemed almost naked on account of the small size of the bracts. Dr. J. Nieuwland has furnished me such excellent descriptions of Greene's type specimens as to give me assurance that certain other sheets of the same collections bearing the same numbers as Greene's type sheets represent the same forms. Each of Greene's plants has thick leaves pubescent upon both faces and densely glaucous beneath. In middle peninsular Florida, there occurs a *Polycodium* the leaves of which are in-

[1] Fl. Bor.-Am. **1**: 328. 1803.
[2] Pittonia **3**: 249. 1897.
[3] Leaflets Bot. Obs. **2**: 226. 1912.

variably green beneath, but it is associated with one (*P. oliganthum* Greene) with smaller and more pointed leaves which are consistently glaucous beneath. Both as a rule have foliaceous bracts, black fruit maturing in mid-summer and scattered over the plant, in place of being clustered on branchlets which are largely destitute of leaves, and leaves pubescent on both surfaces —characters common to certain species of the pine region of the southeastern coast. The plant with the leaves green beneath grows in the hammocks; the other is restricted to the high sandy pine woods and the "scrub." A description of this plant follows:

Polycodium quercinum sp. nov. A shrub 1–1.5 m. high. Leaves rather thin, rounded or obtuse at the apex, oval, obovate or elliptic, 2.5–4.5 cm. long, 1–2 cm. wide, mostly rounded at the narrowed base, rarely subcordate, the margin ciliate, and sometimes slightly revolute, especially after midsummer on exposed or dry sites, above dark green and sparingly pubescent with short spreading hairs, beneath yellowish green, and with short spreading grayish pubescence over the entire lower surface as well as on the short petiole. Twigs slender, pubescent with short spreading, often matted, hairs. Flowers, appearing about the middle of April, small, about 4 mm. long with acute divisions to the corolla, from the axils of the leaf-like bracts on slender, nodding, rarely 1 cm.-long pubescent pedicels. Fruit black or dark purple-black, glossy, 7–12 mm. thick, not large for the genus, sparingly pubescent.

Type: Abundant in hammocks near Lynne, Marion County, Florida, W. W. A. April 1923, July 1926.

FRAXINUS CATAWBIENSIS Ashe.[4] On account of the infrequency of its occurrence in middle North Carolina, the region from which originally reported, it was eventually assumed that this ash was a hybrid, although it was not possible with accuracy to fix its parentage. In recent years, however, it has been noted frequently in the foothills region of northeastern Georgia. Its characters seem well fixed. Specimens of *Fraxinus profunda* Bush or *F. biltmoreana* Bead., species which might be considered as one of the parents, are not known to occur in this immediate region. It seems desirable to regard it as an infrequent and rather local species related to *F. profunda* by its slightly flattened seed, but lacking its large calyx; resembling *F. biltmoreana* in its pubescence and in the pale often glaucescent

[4] Bot. Gaz. 33: 230. 1902.

lower surface of the foliage, but lacking its stout terete seed; approaching *F. americana* in the general shape of its fruit and in the pale lower surface of the leaflets, but separated by the velvety and persistent pubescence on the twigs, and the slightly flattened and not terete body to the seed. It has been noted as far south as Dawsonville (5 miles south, on the Atlanta road), Dawson County, Georgia, and seems to be more abundant in Rabun, White and Habersham Counties, Georgia, than elsewhere.

× **Schmaltzia Ashei** hyb. nov. *S. Ashei* Small, Fl. S. U. S. Ed. 1. 729. *Rhus caroliniana* Ashe, Bot. Gaz. **20**: 548. 1895. This species was based upon herbarium specimens collected from wild plants. Later it was cultivated. Seedlings from these cultivated plants varied greatly, some approaching *S. glabra* (L.) Small and others *S. Michauxii* (Sarg.) Small. It is believed that it is a hybrid between these plants and should be dropped as a species.

QUERCUS ARKANSANA CAPUT-RIVULI Ashe.[5] During the summer of 1926, specimens of this local tree were noted in Bibb County, Ala., at and near Woodfin Tucker's store; in Hale County, Ala., near Havana; in Tuscaloosa County, Ala., along the highway north of Moundsville and about eight miles south of Tuscaloosa, growing in each case upon sandy banks near small water courses. Two trees at Woodfin Tucker's store, probably planted, were 16 and 18 inches in diameter, 45 and 50 feet high, respectively. The deliquescence of the prominent veins near the margin of the leaf and consequent prevailing absence of awns was characteristic of the leaves in each of these localities, just as it is in trees growing in West Florida and Georgia.

PADUS CUTHBERTII Small. This well marked species, which has heretofore been reported only from the vicinity of Augusta, Georgia, was found (W. W. A., 1923, 1926) growing around stream heads at Crestview and Mossyhead, West Florida.

In connection with the pubescent summer shoots of certain species of *Tilia* which have glabrous spring shoots, it may be of interest to note that many summer shoots of *Tilia heterophylla* and its varieties were collected during the summer of 1926 in Georgia, and Alabama. These were all glabrous, thus con-

[5] Jour. Elisha Mitchell Soc. **40**: 44. Aug. 1924.

firming previous observations upon the summer shoots of this species.

CASTANEA MARGARETTA forma **Dormonae** form. nov. This is a rare form which occurs in middle Louisiana, having three large nuts to the involucre in place of one. If this form occurred within the distribution of chestnut it would doubtless be regarded as a hybrid or as having a chestnut strain. Chestnut is not known to occur, however, within 350 miles of Natchitoches Parish, Louisiana, the station for this form, which was collected there by Miss Caroline Dormon. In its long pubescent twigs, and the densely set and elongated spines on the fruit, this form approaches variety *arcuata* Ashe of the Gulf Coast of Louisiana and the "Big Thicket" region of Texas.

× **Hicoria ludoviciana** hyb. nov. *H. aquatica* (Michx. f.) Brit. × *H. Buckleyi* var. *arkansana* (Sarg.) Ashe. This interesting hybrid, which has been collected and studied by Miss Caroline Dormon, of Natchitoches Parish, Louisiana, where it is found, has the foliage, buds, bark and general aspect of *H. Buckleyi*. The fruit, however, is compressed, the husk very thin, the nut wrinkled, particularly at the base, the shell though not brittle, so thin that it can be crushed between the fingers, and the very large kernel is much wrinkled and bitter.

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Kieffer pear seedlings and fire blight resistance

H. E. Thomas

The Kieffer pear is a hybrid between the oriental pear (*Pyrus serotina* var. *culta* Rehder) and the European species (*P. communis* var. *sativa* Linn.), presumably the variety Bartlett. The latter parent represents the more desirable fruit characters of the European pear, and the former exhibits a high degree of resistance to the fire blight disease caused by *Erwinia amylovora* (Burrill) Comm. S.A.B. Kieffer is intermediate between its parents in fruit quality and blight resistance. Since fire blight is still an important limiting factor in the cultivation of the pear, it has seemed desirable to make further selections from hybrids of the Kieffer type. This somewhat heterodox method was adopted because of its obvious advantage in quickly placing a relatively large number of individuals under experiment. Accordingly, a considerable quantity of Kieffer seed was obtained in 1921 from open-pollinated trees on the University farm at Ithaca, N. Y. These were planted in April, 1921; and in 1924, 774 seedlings had survived the hazards of transplanting, rodents, and low temperatures.

A further departure from the usual fruit breeding practice was then made in an effort, by repeated inoculation, to eliminate at an early age those individuals which were susceptible to blight. Thus it will be necessary to bring only a small part of the original population up to fruiting age. During the seasons of 1924, 1925, and 1926, all the seedlings were inoculated at least five times with the fire blight organism. Many of them received ten or more inoculations. Cultures were used from both pear and apple and from widely separated localities. In August, 1924, 177 of the 774 trees were blighted. In October, 1926, 368 of the 679 remaining trees had become more or less affected.[1] These

[1] Four arbitrary classes of resistance were set up. Class 0 was completely resistant; class 1, blight checked within the current season's growth; class 2 was killed back to the second year growth; in class 3 the organism had penetrated into the wood of preceding seasons frequently killing the entire trees.

In the records given above classes 1, 2, and 3 are included in the blighted group, while in table 1, classes 0 and 1 are contrasted with the relatively susceptible classes 2 and 3.

figures are by no means final, since infection is closely related to fluctuating growth conditions of the trees. For example, from the records of 1924 in parts of rows on poorer soil, 6, 9, and 10 per cent of the trees were infected, while in parts of the same rows a few rods distant but in better soil, 20, 25, and 26 per cent of the trees were blighted.

The seedlings showed an extremely wide variation in most of the characters which were examined. The question then arose as to whether there existed any parallel between blight resistance and external characters of the tree. A preliminary examination of the form, color and other characters of the various parts of the tree disclosed no striking parallel between resistance and any of the characters studied. However, several of the more definite characters when examined in greater detail were found to be more frequently associated with resistance than were their corresponding characters (see table 1). The relation

TABLE I

Some tree characters associated with resistance to fire blight

CHARACTER		RESISTANT	SUSCEPTIBLE	RATIO
Branchlets	Stout	107	58	100 : 54
	Slender	80	117	100 : 146
Color of branches 2–4 years old	Red to reddish brown	73	38	100 : 52
	Green to grayish green	69	74	100 : 107
Spininess	Spiny	147	147	100 : 100
	Smooth	37	30	100 : 81
Lenticel size	Large	12	16	100 : 133
	Small	24	35	100 : 145
Leaf breadth in relation to length	Broad	123	114	100 : 92
	Narrow	43	48	100 : 111
Leaf serration	Deep	21	26	100 : 123
	Not deep	61	47	100 : 77

is not assumed to be other than an accidental parallel and is not sufficiently constant to provide any notable aid in selection for resistance. It is interesting, however, that the character associated with greater resistance may be one rather of the susceptible than of the resistant parent. This is true of the reddish color of bark, this character being among those most frequently associated with resistance. On the other hand, the spiny character of the susceptible parent is found quite as often in the resistant as in the susceptible class. Support is thus given to the expectation that resistant individuals of this population may show the desirable tree and fruit characters of the *P. communis* parent.

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INDEX TO AMERICAN BOTANICAL LITERATURE
1920–1927

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